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09-05-2008	Final Report	15-Aug-2006 - 14-Feb-2008

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Rapid Altitude Acclimation Final Report

5a. CONTRACT NUMBER
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5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
6D10AC

6. AUTHORS
Martha Tissotvanpatot, David Irwin, Robert Gotshall, Karyn Hamilton

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Colorado - Health Science Center
Fitzsimons Bldg. 500
Mail Stop F428, PO Box 6508
Aurora, CO 80045 -0508

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

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ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
51047-LS-DRP.2

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; Federal purpose rights

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The goal of this seedling was to determine whether cell-free hemoglobin (HBOC) solutions could enhance oxygen carrying capacity of blood during exposure to high altitude (14,000 ft and above). The outcome of interest was increased altitude exercise performance secondary to improved oxygen carrying capacity of blood. Male Sprague Dawley rats were habituated to treadmill running, then evaluated on time to fatigue at base altitude (1500 m) and then again at 4300 m. Running times were reduced for rats at altitude. Treatment with HBOC worsened running times by ~ 50%, despite increased oxygen carrying capacity of blood. This led to an investigation as to cause and to possible interventions. Additionally, novel tissue

15. SUBJECT TERMS
polymerized bovine hemoglobin, high altitude, nitric oxide, hypoxia inducible factor, vascular endothelial growth factor

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			David Irwin
U	U	U	SAR		19b. TELEPHONE NUMBER 303-315-8628

Report Title

Rapid Altitude Acclimation Final Report

ABSTRACT

The goal of this seedling was to determine whether cell-free hemoglobin (HBOC) solutions could enhance oxygen carrying capacity of blood during exposure to high altitude (14,000 ft and above). The outcome of interest was increased altitude exercise performance secondary to improved oxygen carrying capacity of blood. Male Sprague Dawley rats were habituated to treadmill running, then evaluated on time to fatigue at base altitude (1500 m) and then again at 4300 m. Running times were reduced for rats at altitude. Treatment with HBOC worsened running times by ~ 50%, despite increased oxygen carrying capacity of blood. This led to an investigation as to cause and to possible interventions. Additionally, novel tissue harvesting in hypoxic conditions has permitted the unique evaluation of cell markers of hypoxia, such as HIF1-alpha. Hemodynamic data showed that in, awake rats, marked reductions in cardiac output, elevated blood pressure, and increased vasoconstriction to HBOC infusion. Use of LNAME provided information to suggest that HBOC scavenges NO. Managing the NO scavenging with a PDE-5 inhibitor or S-nytrosylated HBOC resulted in improved exercise performance (~40%) at high altitude. This study suggests that HBOC therapy may be used to enhance exercise performance at high altitude if NO scavenging is managed.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

One pending.

Number of Papers published in peer-reviewed journals: 1.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

original title: HEMOGLOBIN BASED OXYGEN CARRIERS DECREASE OXYGEN DELIVERY DURING NORMOXIA AND ACUTE HYPOXIA-

Revised title: Polymerized bovine hemoglobin decreases oxygen delivery during normoxia and acut hypoxia in the rat.

Number of Manuscripts: 1.00

Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED
Robert Jacobs	0.50
FTE Equivalent:	**0.50**
Total Number:	**1**

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Robert Gotshall	0.20	No
Eric Monnet	0.10	No
Martha TissotvanPatot	0.20	No
David Irwin (did not need salary suppo	0.00	No
Karyn Hamilton (did not need salary su	0.00	No
Tim Hackett (Did not need salary supp	0.00	No
FTE Equivalent:	**0.50**	
Total Number:	**6**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
Brian Piteo	0.50
FTE Equivalent:	**0.50**
Total Number:	**1**

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	1.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	1.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	1.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME	
Robert Jacobs	
Total Number:	**1**

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED	
Benjamine Foreman	1.00	No
Molly White	1.00	No
FTE Equivalent:	**2.00**	
Total Number:	**2**	

Sub Contractors (DD882)

Inventions (DD882)

I. Background

This is the final report and recommendations by the "research group affiliated with University of Colorado Health Science Center and Colorado State University for the seedling grant titled "Rapid Altitude Acclimatization" **August 15, 2006-February 14, 2008**. The objective of this study was to investigate the feasibility of enhancing exercise performance at high altitude utilizing hemoglobin based oxygen carriers (HBOC).

II. **Statement of Work:** The project consisted of two primary hypothesis**: 1)** Determining if treatment with the HBOC Oxyglobin® would increase arterial oxygen content and enhance exercise performance at high altitude; **2)** Determining if treatment with a combination of Oxyglobin and a nitric oxide donor would be more efficacious at increasing oxygen delivery and enhancing exercise performance at high altitude vs. Oxyglobin® alone. Sub aims under each hypothesis investigated HBOC effect on pulmonary vascular leak, vascular endothelial growth factor (VEGF) and VEGF receptor soluble flt-1.

III. To meet our **statement of work** objectives a rat exercise model was developed in which to evaluate HBOC solutions to enhance oxygen carrying capacity of blood during exposure of animals to high altitude (14,000 ft and above). The outcome of interest was increased altitude exercise performance secondary to improved oxygen carrying capacity of blood. In addition the following parameters were analyzed 1) cardiovascular and hemodynamic responses; 2) vascular leak; 3) hypoxia-induced intracellular pathways including hypoxic inducible factor (HIF) and vascular endothelial growth factor (VEGF) and metabolomic markers.

IV. Results:

Primary aim 1: Would Treatment with the HBOC Oxyglobin® increase arterial oxygen content and enhance exercise performance at high altitude.

Hemodynamic studies in, hypoxic, conscious rats demonstrated marked reductions in cardiac output (Figure 1), elevated blood pressure, and increased vasoconstriction to HBOC infusion. Use of NLA to block nitric oxide (NO) provided information to suggest that HBOC scavenges NO, resulting in negative cardiovascular changes; thereby, offsetting any increased oxygen carrying capacity of HBOC (Figure 1).

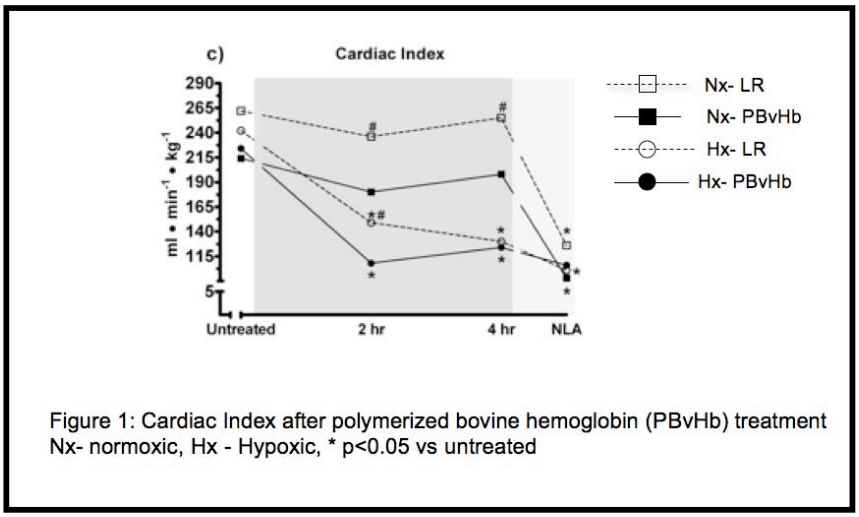

Figure 1: Cardiac Index after polymerized bovine hemoglobin (PBvHb) treatment
Nx- normoxic, Hx - Hypoxic, * p<0.05 vs untreated

Exercise performance studies: Male Sprague Dawley rats were habituated to treadmill running, then evaluated on time to fatigue at base altitude (1500 m) and then again at 4300 m. Running times were reduced for rats at altitude. Treatment with HBOC worsened running times by about half (50%), despite increased oxygen carrying capacity of blood. This led to an investigation as to cause and to probable interventions.

hypoxia-induced intracellular pathways:
Hypoxia inducible factor (HIF): Novel tissue harvesting in hypoxic conditions has permitted the unique evaluation of cell markers of hypoxia, including HIF1-alpha. HBOC treatment decreased HIF-1α in the kidney and brain after a 4 h exposure to a simulated high altitude of 18,000 ft. This is a significant observation when evaluated with hemodynamic and oxygen delivery data that suggested oxygen delivery was decreased with HBOC infusion. We explored whether or not nitric oxide scavenging by HBOC may explain this phenomena. HIF-1a was reduced approximately the same amount when a nitric oxide synthase (NOS) inhibitor was administered (Figure 2). This would suggest that NO scavenging by HBOC does have an effect on HIF induction, but under these conditions whether or not this is beneficial is yet to be determined.

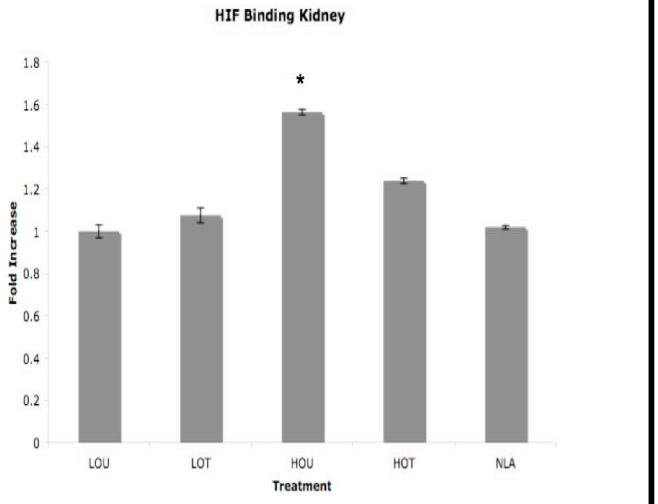

Figure 2. Hypoxic inducible factor(HIF) DNA binding in the kidney. LOU- low altitude untreated; LOT- Low altitude PBvHb treated; HOU-high altitude untreated;HOT- High altitude PBvHb treated; NLA- High altitude treated with the NOS inhibitor NLA. PBvHb- polymerized bovine hemoglobin. *p<0.05 vs all other treatments

Vascular endothelial growth factor (VEGF)
Vascular endothelial growth factor (VEGF) and VEGF receptor (sflt-1): Treatment with HBOC increased plasma concentrations of VEGF in all animals (Figure 3). Plasma concentrations of total sFlt-1 significantly increased following, both, hypoxic exposure and HBOC administration. A Pearson's Correlation was run to examine the relationship between changes in plasma concentrations of unbound VEGF and plasma concentrations of total sFlt-1. No significant relationship was observed. A significant increase in plasma concentrations of unbound VEGF following HBOC administration despite an attendant rise in total sFlt-1 plasma concentrations is surprising and warrants further Investigation.

Pulmonary vascular leak: Pulmonary vascular leak was increased with HBOC treatment and this was not associated with increased arterial pressure, but associated

with increased circulating VEGF. This is an important observation in how HBOC are modulating VEGF. HIF-1 a is an important transcription factor of VEGF, but as previously described the HBOC used in this study decreased HIF-1. It's known that inflammatory mediators can induce VEGF. Thus, to reconcile the paradoxical relationship induced from HBOC infusion between HIF and VEGF further investigation of HBOC-induced inflammation is warranted.

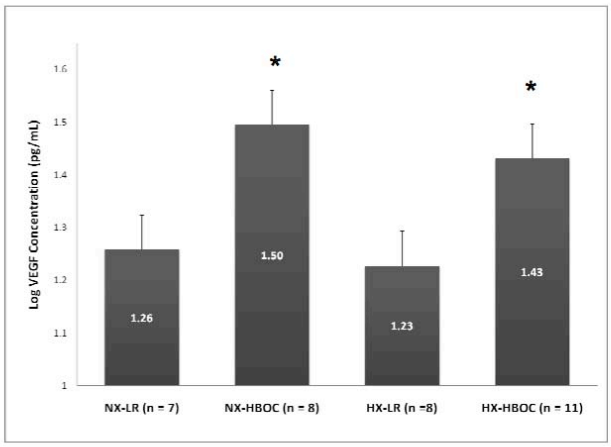

Figure 3. Polymerized Bovine Hemoglobin treatment (HBOC) effect on VEGF. (n values). LR- lacted ringers (control) treated, Nx- normoxic, Hx- Hypoxic. * p<0.05 vs untreated

Primary aim 2: Treatment with a combination of Oxyglobin and a nitric oxide donor would be more efficacious at increasing oxygen delivery and enhancing exercise performance at high altitude vs. Oxyglobin® alone.

Hemodynamic effects: As a strategy to reduce NO scavenging by HBOC, or to provide enhanced NO responsiveness, in order to improve cardiovascular function during HBOC infusion; thereby, enhancing oxygen delivery and running times at altitude we s-nitrosylated HBOC to delivery NO. Compared to HBOC (Oxyglobin) alone, an infusion of treated (i.e S-nitrosylatated), and untreated Oxglobin preserved normal hemodynamic and cardiac function (Figure 4). This translated to increased oxygen delivery in rats exposed to high altitude. Additionally, compared to Oxyglobin and Oxyglobin + PDE-5 inhibitor also increased the cardiac index (Figure 5). This data supports our hypothesis that

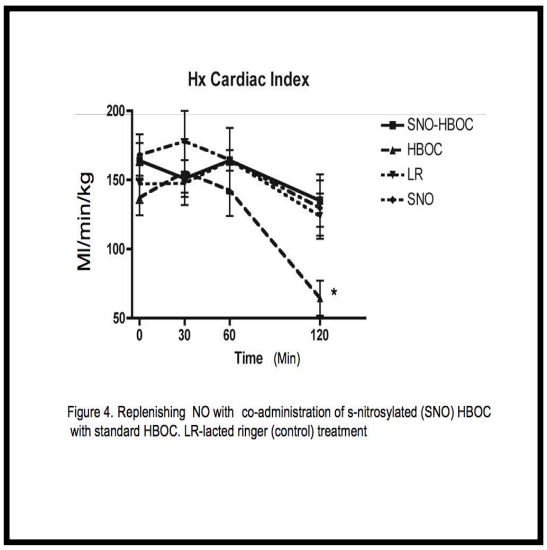

Figure 4. Replenishing NO with co-administration of s-nitrosylated (SNO) HBOC with standard HBOC. LR-lacted ringer (control) treatment

Oxyglobin scavenges NO and that increasing NO availability (i.e. SNO) or enhancing the signal (i.e. inhibiting PDE-5) reduces vasoactive side effects of HBOC

Exercise performance: Running times were increased from vehicle control rats at altitude (~40%). As a proof of concept that increasing NO availability would off-set HBOC induced side effects PDE-5 inhibitors were giving simultaneously with HBOC. This treatment combination (HBOC+PDE-5 inhibitor) also increased exercise performance at high altitude.

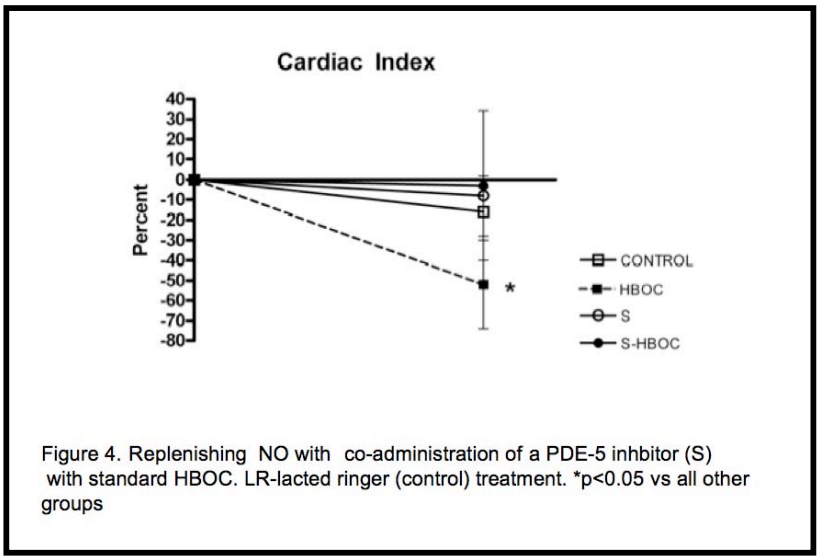

Figure 4. Replenishing NO with co-administration of a PDE-5 inhbitor (S) with standard HBOC. LR-lacted ringer (control) treatment. *p<0.05 vs all other groups

V. Recommendations: In this seedling grant we have shown that infusion with an hemoglobin based oxygen carrier, Oxyglobin® (1.3g/kg), increases arterial oxygen content approximately 10-15% above normal values. However, we have also demonstrated the darker side of HBOC infusion. Due to NO scavenging HBOC induced a dramatic decrease in cardiac index and this resulted in a net decrease in oxygen delivery. Our results also clearly demonstrate that if one offsets the NO scavenging by either increasing nitric oxide synthase via a PDE-5 inhibitor or chemically attaching a NO to the hemoglobin (SNO), cardiac output is maintained and oxygen delivery is increased, and exercise performance at high altitude is modestly improved.

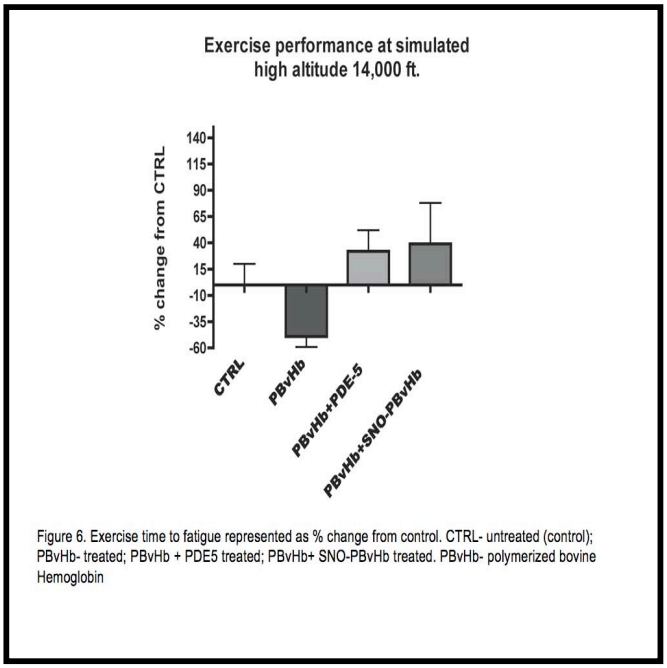

Figure 6. Exercise time to fatigue represented as % change from control. CTRL- untreated (control); PBvHb- treated; PBvHb + PDE5 treated; PBvHb+ SNO-PBvHb treated. PBvHb- polymerized bovine Hemoglobin

Thus, our data supports the theory and feasibility that HBOC may improve soldier performance at high altitude. However, For HBOC to improve high altitude exercise performance in soldiers the NO scavenging effect must be managed. We believe that this can be managed by the co-administration of an NO donor. The burning questions that our research leaves un-addressed are **1)** the optimal dose of SNO-

hemoglobin or PDE-5 inhibitor to achieve the a maximum benefit; **2)** an optimal HBOC P_{50} and **3)** an optimal cross link material/method. Finally, we believe that answering these questions are of importance not only to the military, but also for the clinical relevancy in the development of HBOC in general.

VII Report of inventions and subcontracts and Federal transaction report.

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
OMB No. 9000-0095
Expires Aug 31, 2001

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THIS ADDRESS. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT (X one)
University of Colo.Hlth Sci Ctr	W911NF-06-0318	University of Co.Hlth Sci.Ctr	W911NF-01-0318	a. INTERIM [ ] b. FINAL [X]
b. ADDRESS (Include ZIP Code)	**d. AWARD DATE** (YYYYMMDD)	**b. ADDRESS** (Include ZIP Code)	**d. AWARD DATE** (YYYYMMDD)	**4. REPORTING PERIOD** (YYYYMMDD)
Fitzsimons Building 500,MailStop F-428 P.O.Box 6508, Aurora, Co. 80045-0508	060815	Fitzsimons Building 500,Mailstop F-428 P.O.Box 6508,Aurora, Co.80045-0508	060815	a. FROM 060815 b. TO 080214

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR (If "None," so state)

NAME(S) OF INVENTOR(S) (Last, First, Middle Initial) a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS (X) d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER (X) e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
	NONE							

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR

(1) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(2) (a) NAME OF INVENTOR (Last, First, Middle Initial)	g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
NONE		(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER	NONE	
(c) ADDRESS OF EMPLOYER (Include ZIP Code)	(c) ADDRESS OF EMPLOYER (Include ZIP Code)		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR (If "None," so state)

NAME OF SUBCONTRACTOR(S) a.	ADDRESS (Include ZIP Code) b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES (YYYYMMDD) f.	
			(1) CLAUSE NUMBER	(2) DATE (YYYYMM)		(1) AWARD	(2) ESTIMATED COMPLETION
Colorado State University	Sponsored Programs 408 University Serv Bldg Fort Collins, Co 80523-1644	SPO 58123		0608	Rapid Altitude Acclimation	060815	$146,423.00

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR (Not required if: (X as appropriate)) [ ] **SMALL BUSINESS** or [ ] **NONPROFIT ORGANIZATION**

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL (Last, First, Middle Initial)	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Patricia A. Plummer	Planning/Grants Officer	Patricia Plummer	5/8/08

Approved by Office of Management and Budget, No. 80-R0182

FEDERAL CASH TRANSACTION REPORT

(See instructions on the back. If report is for more than one grant or assistance agreement attach completed Standard Form 272-A)

1. Federal sponsored organizational element to which this report is submitted: U.S. Army Research Acquisition

2. Recipient Organization

Name: UCHSC at Fitzsimons
Office of Grants and Contracts

Number and Street: Fitzsimons Building 500, Mail Stop F-428
P.O. Box 6508

City, State and Zip Code: Aurora, CO 80045-0508

4. Federal grant or other identification number: W911NF-06-1-0318

5. Recipient's account number or identifying number: 2521563-006 FINAL

6. Letter of Credit Number:

7. Last Payment Voucher #:

Give total number for this period

8. Payment Vouchers credited to your account:

9. Treasury checks received (whether or not deposited):

3. FEDERAL EMPLOYER IDENTIFICATION NO. ⟹ 84-6000555

10. PERIOD COVERED BY THIS REPORT

FROM (month, day, year): 01/01/08

TO (month, day, year): 02/14/08

11. STATUS OF FEDERAL CASH (See specific instructions on the back)

a. Cash on hand beginning of reporting period	$ 57,978.57
b. Letter of credit withdrawals	0.00
c. Treasury check payments	$ 0.00
d. Total receipts (Sum of lines b and c)	$ 0.00
e. Total cash available (Sum of lines a and d)	$ 57,978.57
f. Gross disbursements	$ 68,124.57
g. Federal share of program income	0.00
h. Net disbursements (Line f minus line g)	$ 68,124.57
i. Adjustments of prior periods	0.00
j. Cash on hand end of period	$ (10,146.00)

12. THE AMOUNT SHOWN ON LINE 11J ABOVE REPRESENTS CASH REQUIREMENTS FOR THE ENSUING Days

13. OTHER INFORMATION

a. Interest income	$
b. Advances to subgrantees or subcontractors	$

14. REMARKS (Attach additional sheets of plain paper, if more space is required)

15. CERTIFICATION

I certify to the best of my knowledge and belief that this report is true in all respects and that all disbursements have been made for the purpose and conditions of the grant or agreement

AUTHORIZED CERTIFYING OFFICIAL

SIGNATURE: Cathy J. Hatter

DATE REPORT SUBMITTED: 3/24/08

TYPED OR PRINTED NAME AND TITLE:
Cathy J. Hatter
Accountant

TELEPHONE (Area Code, Number, Extension)
(303) 724-0279

THIS SPACE FOR AGENCY USE